Time-Dependent Shifts in Fermentation Endproduct Ratios: A Source of Bias in Estimation of Digestion Kinetics Via In Vitro Measurement of Gas Production

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Introduction

Continuous online measurement of gas production in sealed vials has the potential to determine, with greater ease than conventional methods, the kinetics of fermentation of forage materials by ruminal microorganisms. As a result, gas production has been used to compare in vitro kinetics of digestion of various feedstuffs. However, direct calculation of substrate consumption from gas production data is not feasible for a number of reasons. For example, differences in the composition among feeds result in different ratios of fermentation endproducts, which, in turn, affects gas production. A relationship between gas yields and fermentation endproducts has been described by Beuvink and Spoelstra:

mL gas production = V_m [acetate] + 2(V_m) [butyrate] + 0.87 V_m [total VFA], [1]

where V_m is the molar gas volume; these workers routinely measure gas production at the end of the fermentation to provide qualitative information on the deviation of observed gas production from theoretical values.

Digestion kinetics based on gas production can be biased by changes in the proportions of fermentation endproducts that may accompany digestion in batch culture. The purpose of this study was to determine the extent of change in the formation of endproducts during fermentation of three substrates and to quantify the extent to which these changes can bias the theoretical gas yields from a fixed amount of substrate over the course of an in vitro digestion experiment.

Methods

Concentrations of VFAs were determined over time during the fermentation of three substrates: corn silage, alfalfa hay, and microcrystalline cellulose; the last substrate, while not a direct feedstuff for ruminants, is often used as a standard substrate in in vitro gas production experiments. Fermentations were conducted under a CO_2 atmosphere in 60 ml serum vials that contained 80 mg of substrate and 6 ml of a pre-reduced, modified Goering and Van Soest buffer. After warming of vials to 39 °C, vials were inoculated with 4 ml of ruminal inoculum from a cow fed alfalfa hay; the inoculum was prepared by squeezing rumen fluid through four layers of cheesecloth followed by wetting of the solids with an equal volume of Goering/Van Soest buffer and squeezing this through the cheesecloth. Vials were sealed with butyl stoppers and aluminum crimp seals, then incubated at 39 °C. Pairs of vials containing each substrate were removed at intervals, and VFA concentrations determined by HPLC.

Results

The shift in the molar ratios of acetate and butyrate during the fermentations are shown in Fig.1. Shifts in the molar ratios of propionate were also observed but were excluded from the figure because propionate production does not contribute to net production of gas. The molar ratios of products for alfalfa fermentation were

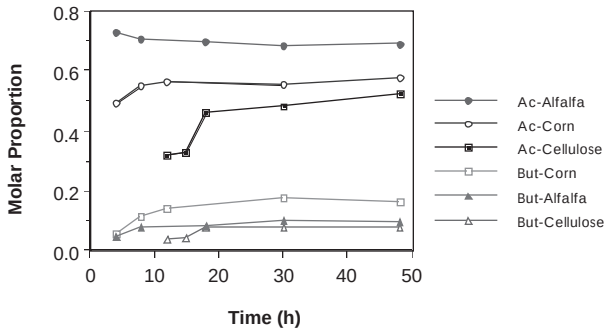

Figure 1. Shifts in the molar proportion of acetate and butyrate during the in vitro fermentation of alfalfa hay, corn, and microcrystalline cellulose.

essentially constant throughout the incubation period. However, both corn silage and microcrystalline cellulose showed marked increases in the molar proportions of acetate and butyrate during the course of the fermentations.

To determine the potential bias in gas production resulting from these time-dependent changes in gas production, the fermentation product data at different times were normalized on a molar basis using a modification of equation [*1*]:

$$Y_t = X_{acetate} + 2\,X_{butyrate} + 0.87 \qquad [2]$$

where Y_t is the relative gas production per unit substrate at time *t* (estimated from equation [*1*]), and $X_{acetate}$ and $X_{butyrate}$ are molar proportions of acetate and butyrate, respectively.

Bias in gas production estimated at different times using fixed, endpoint (48 h) values of VFA proportions were then calculated as:

$$Bias = (Y_t - Y_{48h}) / Y_{48h} \qquad [3]$$

where Y_{48h} is estimated gas production per unit substrate based on the proportions of VFAs at 48 h. A positive value indicates an underestimate of substrate consumption (i.e., an overestimate of gas production relative to that expected for fermentation endproduct ratios determined at the end of the incubation) . Results from these calculations are shown in Fig. 2. Bias introduced by changes in fermentation endproduct ratios was greatest at early time points and reached values of 20% or more. Because measurements during the first 24 h have the greatest effect on determining digestion rate and lag time, changes in VFA patterns during the fermentation must be accounted for to prevent biased estimates of digestion kinetics.

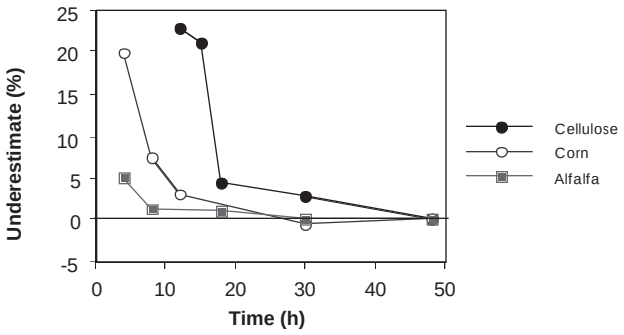

Figure 2. Time-dependent bias in the estimation of digestion of three substrates from gas production measurements. Bias was calculated on the basis of differences in the endproduct ratios at the indicated times relative to the ratios at the 48 h endpoint.

Conclusions

Changes in the ratios of fermentation endproducts over time complicate determination of substrate consumption from gas production data. In this experiment, bias in gas production introduced by assuming fixed endproduct ratios is small for alfalfa but may result in an underestimation of 20% or more for corn and cellulose early in their fermentations. Further experiments are needed to determine if the shifts in fermentation products observed for corn and cellulose were due to characteristics of the feed or to the use of inocula from an alfalfa-fed cow. Nevertheless, proper corrections of gas production data to more accurately reflect substrate consumption will require separate determinations of VFA profiles over time for each substrate of interest.